

Copyright  
by  
Robert Henry Gregg  
2018

**The Report Committee for Robert Henry Gregg  
Certifies that this is the approved version of the following Report:**

**Influence of ischemia on the discharge rate in motor units  
during a sustained submaximal contraction**

**APPROVED BY  
SUPERVISING COMMITTEE:**

---

Lisa Griffin, Supervisor

---

Jody Jensen

**Influence of ischemia on the discharge rate in motor units during a  
sustained submaximal contraction**

**by**

**Robert Henry Gregg**

**Report**

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

**Master of Science in Kinesiology**

**The University of Texas at Austin**

**May 2018**

## **Abstract**

### **Influence of ischemia on the discharge rate in motor units during a sustained submaximal contraction**

Robert Henry Gregg, M.S. Kin

The University of Texas at Austin, 2018

Supervisor: Lisa Griffin

Motor unit discharge patterns were observed under ischemic and non-ischemic conditions in the tibialis anterior during two sessions approximately 48 hours apart. Ten subjects completed three 5-second maximum voluntary contractions [MVCs) followed by a prolonged, submaximal, isometric contraction to induce fatigue, and three additional MVCs. This fatigue trial was completed under both ischemic and non-ischemic conditions. For the ischemic condition, a pressure cuff was placed above the knee, inflated to 180 mmHg, and arterial occlusion verified via Doppler ultrasound. During the fatigue task, a constant force of 20% MVC was maintained until endurance time. Single motor unit activity was recorded with intramuscular fine wire electrodes. Endurance time during the ischemic fatigue task ( $3.71 \pm 0.58$  min) was significantly less than the non-ischemic fatigue task ( $9.11 \pm 0.56$  min). Both tasks showed a significant decline in motor unit discharge rate. There was greater variability in the discharge rate during ischemic

compared to non-ischemic conditions. Time to minimum discharge rate was greater during ischemic conditions. Overall, ischemic conditions resulted in decreased endurance time, greater rate of decline in discharge rate, and greater variability in discharge rate of motor units.

## Table of Contents

List of Figures .....	vii
<b>1. Introduction.....</b>	<b>1</b>
<b>2. Methods.....</b>	<b>3</b>
2.1 Participants.....	3
2.2 EMG Recording.....	3
2.3 Experimental Protocol .....	3
2.4 Data Analysis .....	5
2.5 Statistical Analysis.....	6
<b>3. Results .....</b>	<b>7</b>
3.1 Individual-level variance .....	7
3.2 Motor unit discharge patterns .....	7
3.3 Coefficient of variation .....	8
<b>4. Discussion.....</b>	<b>9</b>
<b>5. Conclusion .....</b>	<b>11</b>
References.....	12

## List of Figures

- Figure 1: The bottom trace shows recording from the Doppler ultrasound. The blood flow is indicated by the initial waves; however a flat line afterwards indicates that the blood flow was occluded. The torque output trace shows an initial baseline followed by torque generated at 20% of maximum voluntary contraction. The top trace shows the intramuscular EMG recording. ....5
- Figure 2: Model estimates for percent of initial discharge rate for ischemia and non-ischemic conditions. The bands are  $\pm$  standard error. ....7
- Figure 3: Model estimates for discharge rate coefficient of variation for ischemia and non-ischemic conditions. The bands are  $\pm$  standard error.....8

## **1. INTRODUCTION**

Blood-flow-restriction (BFR) training has become a popular training modality in recent years. There are similar gains in cross-sectional area (CSA) of a muscle during resistance training when utilizing low loads (~20% 1-RM) combined with BFR, compared to higher loads (~70% 1-RM) in normal conditions [Lixandrao et al., 2017; Loenneke and Pujol 2009; Takarada et al., 2000]. Using lower loads decreases the strain on joints, and may be particularly useful in aging populations, or individuals recovering from surgery. With an aging population and over 950,000 knee arthroscopy surgeries performed in the US [Kim et al., 2011], there is a need for effective interventions to improve or regain function. Following a BFR training protocol, elderly patients have seen increases in strength and functional ability [Abe et al., 2010]. Individuals recovering from knee arthroscopy showed almost twice the gains in knee extension/flexion strength, as well as improvements in stair ascent time, compared to a control group [Tennent et al., 2017]. Thus, BFR training may be an effective tool for clinical rehabilitation [Hughes et al. 2018].

During BFR, muscles fatigue at a faster rate than under normal conditions [Wernbom et al., 2006], due to restriction of metabolic by-products of the muscle caused by the ischemic conditions induced. Bigland-Ritchie et al. [1986] proposed that fatigue in ischemia is caused by a peripheral reflex inhibiting the central nervous system (CNS), since firing rates of motor neurons recover after three minutes under normal conditions but remain depressed when the muscle is held ischemic. This reflex inhibition was supported by studies showing a decrease in the H-reflex amplitudes, with little change in the M-waves under ischemic conditions [Garland and McComas, 1990; Garland 1991].



Mean discharge rates of single motor units have been shown to decline following a fatiguing protocol under non-ischemic conditions [Bigland et al., 1983; Enoka et al., 1989; Garland et al. 1994], as well as under ischemic conditions [Bigland et al., 1986] during sustained maximal contractions, but have been shown to increase in intermittent, submaximal contractions [Moritani et al., 1992]. Increased variability in the discharge pattern of motor unit has been observed following a fatiguing task under non-ischemic conditions [Enoka et al., 1989], but have not been thoroughly investigated under ischemic conditions, particularly throughout the course of a fatiguing trial as opposed to before and after a fatiguing trial. The goal of this study is to observe the effect of ischemia on single motor unit activity during a sustained isometric fatigue task. We hypothesized that under ischemic conditions, motor units would exhibit a greater increase in their discharge variability compared to non-ischemic conditions. This exaggerated decrease in discharge rates will coincide with a sharper decline in mean discharge rate in the motor units under ischemia when compared to non-ischemic conditions.

## **2. METHODS**

### **2.1 Participants**

Four males and four females (age,  $24.8 \pm 1.47$  yr) participated in this study. Twenty motor units (11 non-ischemic, 9 ischemic) were recorded from the tibialis anterior muscle. Participants were free from injury to the right leg and had no history of metabolic or neurological disorders. All participants were informed of the experimental design and risks during an orientation session and signed a written informed consent form. Ethical approval of the study was provided by the Institutional Review Board of the University of Texas-Austin.

### **2.2 EMG Recording**

Prior to placement of the electrodes, the area where they were to be placed was shaved and then cleaned with 70% isopropyl alcohol. An insulated stainless steel fine wire electrode (California Fine Wire Company, Grover Beach, CA) with wire diameter of 0.002mm was inserted into the tibialis anterior of the right leg using a 25-gauge hypodermic needle. The electrode consisted of three wires; one used as the active electrode, another as the reference, and a third wire that was used as a spare. A ground electrode was placed over the medial malleolus of the right leg.

### **2.3 Experimental Protocol**

Participants attended two testing periods, separated by at least 48 hours. Each visit was identical with the exception that one testing period was performed under ischemic conditions. The order of the experiments was randomized. Participants were seated in an adjustable chair with their right knee and ankle fixed at 90°. The right foot was placed on

a metal plate with a strength gauge and strapped to the plate to measure dorsiflexion force. Knee and ankle joints were stabilized with a padded metal bar secured above the knee joint. Prior to insertion of the fine-wire electrodes, three 5-second maximum voluntary contractions (MVCs) were performed, with 10 seconds of rest between. The fine-wire electrodes were then inserted into the tibialis anterior. Participants then performed a fatigue task of holding a 20% isometric MVC until the endurance limit. The endurance limit was defined as either a decrease in sustained torque of 2% or more, or when maximum tremor amplitude exceeded 5% of MVC torque [Garland et al., 1997]. Preliminary tests showed that throughout the fatigue task in the non-ischemic condition, blood flow was not compromised. To induce ischemia for the experimental condition, a pressure cuff was placed above the knee and inflated to 180 mmHg. Blood flow was monitored in the posterior tibial artery using Doppler ultrasound (8 MHz, model 810-A, Parks Medical Electronics Inc. Aloha, USA) to ensure that ischemia was induced by the pressure cuff. Figure 1 shows a single recording from the Doppler ultrasound.

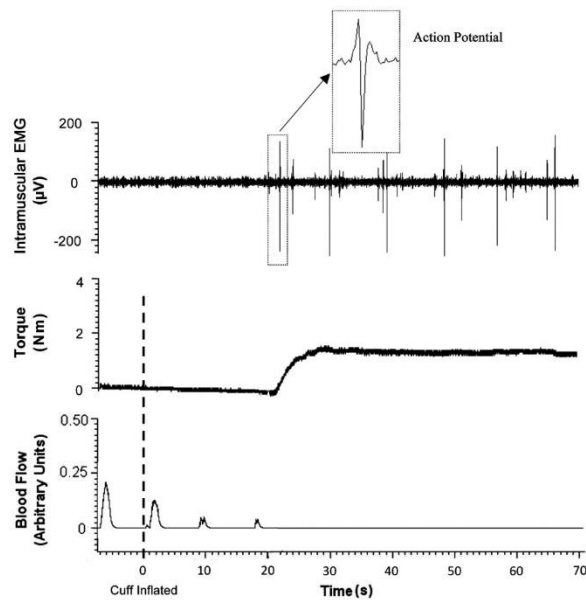


Figure 1

## 2.4 Data Analysis

Intramuscular EMG was pre-amplified and bandpass filtered at 8 Hz-3.12 kHz with a gain of 330 (B&L Engineering, Tustin, CA). The torque and intramuscular EMG were digitized at 1,000 Hz and 20,000 Hz, respectively (Micro 1401 Analog-Digital Converter Cambridge Electronics Design (CED), Cambridge, UK). Dual monitors displayed the target torques and actual torques, as well as the intramuscular EMG recordings taken throughout data collection. All data were analyzed off-line using Spike2 for Windows (Version 5,12CED, Cambridge, England). Individual motor unit potentials were analyzed off-line utilizing Spike2's waveform discrimination system. Mean single motor unit discharge frequency and coefficient of variation was measured every 5% of time-to-task-failure with 5-second time bins. Motor unit discharge frequency was normalized to percent

change from initial discharge rate. All interspike intervals  $\leq 20$  ms and  $\geq 200$  ms were excluded from the analysis.

## 2.5 Statistical analysis

Percent change in motor unit discharge rate and coefficient of variation in motor unit discharge rate were assessed by similar multilevel statistical models. The multiple observations on each motor unit were modeled with a first-order autoregressive variance/covariance structure; the recording of multiple motor units from the same individual was accounted for with an unstructured variance/covariance structure since previous studies have demonstrated that motor unit observations are correlated within an individual [Tenan et al., 2014]. The intraclass correlation was used to confirm the necessity of controlling for subject-level variance. Model estimates were obtained using the restricted maximal likelihood methodology. The respective dependent variable was modeled with both a linear and quadratic term for time, as well as a binary term for ischemia and an interaction of ischemia and time. The two-tailed alpha level for both regressions was 0.05. All data are reported as model estimate  $\pm$  standard error.

### 3. RESULTS

#### 3.1 Individual-level variance

The intraclass correlations for percent change in discharge rate and discharge rate coefficient of variation were 0.13 and 0.37, respectively. Subject-level correlations of this level are sufficient to bias statistical analysis [Tenen et al., 2014] and support the use of a multilevel model approach.

#### 3.2 Motor unit discharge patterns

All motor units analyzed were monitored throughout the entire fatigue task. There were significant main effects for time ( $p < 0.001$ ), quadratic time ( $p = 0.005$ ), and the interaction of time and ischemia ( $p = 0.033$ ). The change in discharge rate was greater in ischemia and the task was terminated prior to recovery of discharge rate (Fig. 2).

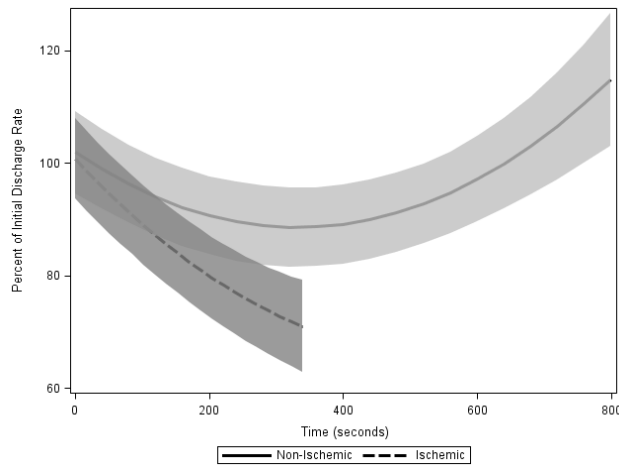


Figure 2

### 3.3 Coefficient of variation

There was a main effect for time on motor unit discharge coefficient of variation ( $p < 0.001$ ). The coefficient of variation rises across the endurance task in both conditions, but the increase is substantially faster in the ischemic condition (Fig. 3).

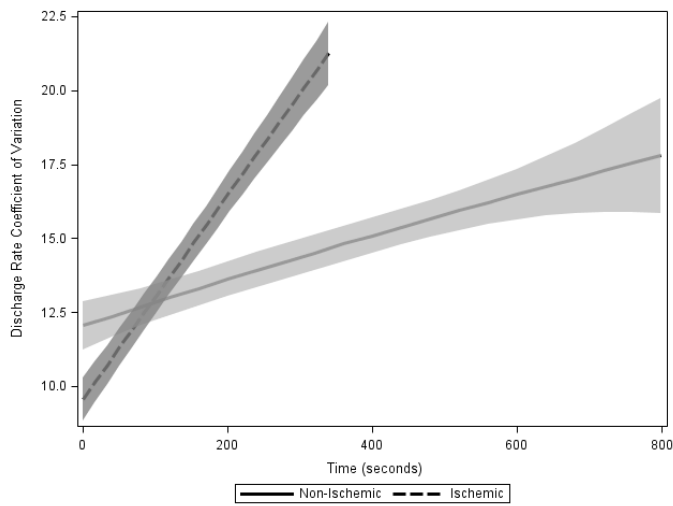


Figure 3

## 4. DISCUSSION

The purpose of this study was to investigate to the effect of ischemia in motor units throughout a fatiguing submaximal sustained contraction. Changes in metabolite concentration can affect the electrical activity of individual motor units. When lactate concentration increases during an ischemic submaximal contraction as oxygen availability decreases, we see greater spike amplitude of individual motor units, and an increase in the firing frequency, which shows the recruitment of additional, high-threshold motor units [Moritani et al., 1992]. In Millet et al. [2009], it was found that there was less fatigability in normobaric hypoxic conditions, than during normoxic conditions where the muscle is occluded. This shows that there is a moderate effect from a lack of oxygen on inhibiting motor drive to the target muscle, but the effect of occlusion on the local environment of the muscle is a greater factor in fatigability. The faccumulation of other metabolites may also factor into changes in fatigability during ischemia. Under conditions where oxygen is limited, there is an increase in hydrolysis of phosphocreatine [Hasseler et al., 1999], leading to an increase of inorganic phosphate ( $P_i$ ). This increase in  $P_i$  may reduce  $Ca^{2+}$  availability in the cell if  $P_i$  precipitates with available calcium when it enters the sarcoplasmic reticulum [Allen et al. 2007]. Other substances that may affect E-C coupling include reduced [ATP] and increased [ $Mg^{2+}$ ] [Dutka and Lamb, 2004]. Fatigue in skeletal muscle may include a slowing of relaxation, which may limit decreases in force production during fatigue even when firing rates decline in a motor unit [Jones et al. 1979]. Decreases in discharge rate may also arise from a prolonged period of after-hyperpolarization [Kernell and Monster, 1982], and central fatigue [Taylor and Gandevia, 2007]. The sharp decline in discharge



rates under ischemia are likely due to lactate concentrations increasing group III and IV afferent feedback to the central nervous system, which decreases voluntary neural drive to the target muscle. These afferents also provide signals to the cardiovascular and respiratory system to increase oxygen delivery to the active muscles. However, due to occlusion of the limb, oxygen delivery is impaired and does not work to mitigate the development of peripheral fatigue in the muscle fiber (Taylor et al., 2016). Under non-ischemic conditions lactate is not as concentrated, and there is less feedback of the group III and IV afferents. This experiment was able to isolate the effect that these muscle afferents have on central fatigue of motor neurons, independent of their mitigating effects on peripheral fatigue of the muscle.

The coefficient of variation of motor unit discharge rate in both conditions increased with fatigue, with greater variability occurring in ischemic conditions. This increase in discharge variability during fatigue has been observed during other submaximal contractions under non-ischemic conditions [Enoka et al., 1989; Garland et al., 1994; Nordstrom and Miles 1991; Pascoe et al., 2014]. Calvin and Stevens [1968] posited that an increase in the variability of discharge rates in motor units in the decerebrate cat are due to an increase in synaptic input. The increased variability during ischemia implies that there is increased neuronal input under occluded conditions compared to non-ischemic conditions. It has been shown that as people age, they exhibit increased variability motor unit discharge, which results in increased fluctuations of force generated during a contraction (Laidlaw et al., 2000).

## **5. CONCLUSION**

This study showed that discharge rates of motor units decrease initially under both ischemic and non-ischemic conditions, however there is recovery in the rate of discharge under non-ischemic conditions that does not occur during ischemia. This is due to differences in muscle afferent feedback inhibiting motor neuron drive. By occluding blood flow to the region, we were able to isolate the effects of the afferents on the development of central fatigue.

## References

- Abe T, Sakamaki M, Fujita S, Ozaki H, Sugaya M, Sato Y, Nakajima T. Effects of low-intensity walk training with restricted leg blood flow on muscle strength and aerobic capacity in older adults. *J. Geriatr. Phys. Ther.* 2010;33:34–40.
- Allen DG, Lamb GD, Westerblad H. Impaired calcium release during fatigue. *J. Appl. Physiol.* 2008;104:296-305.
- Bigland-Ritchie B, Dawson NJ, Johansson RS, Lippold OCJ. Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J. Physiol.* 1986;379:451-459.
- Bigland-Ritchie B, Johansson RS, Lippold OJC, Woods JJ. Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J. Physiol.* 1983;340:335-346.
- Butler JE, Taylor JL, Gandevia SC. Responses of human motoneurons to corticospinal stimulation during maximum voluntary contractions and ischemia. *J. Neurosci.* 2003;23(32):10224-10230.
- Dutka TL, Lamb GD. Effect of low cytoplasmic [ATP] on excitation-contraction coupling in fast-twitch muscle fibres of the rat. *J. Physiol.* 2004;560:451-468.
- Enoka RM, Robinson GA, Kossev AR. Task and fatigue effects on low-threshold motor units in human hand muscle. *J. Neurophysiol.* 1989;62:1344-1359.
- Garland SJ. Role of small diameter afferents in reflex inhibition during human muscle fatigue. *J. Physiol.* 1991;435:547-558.
- Garland SJ, Enoka RM, Serrano LP, Robinson GA. Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction. *J. Appl. Physiol.* 1994;76:2411-2419.
- Garland SJ, McComas AJ. Reflex inhibition of human soleus muscle during fatigue. *J. Physiol.* 1990;429:17-27.
- Hasseler LJ, Hogan MC, Richardson RS. Skeletal muscle phosphocreatine recovery in exercise-trained humans is dependant on O<sub>2</sub> availability. *J. Appl. Physiol.* 1999;86:2013-2018.
- Hughes L, Paton B, Rosenblatt B, Gisene C, Patterson SD. Blood flow restriction training in clinical musculoskeletal rehabilitation: a systematic review and meta-analysis. *Br. J. Sports Med.* 2018;51:1003-1011.

- Hunt JEA, Stodart C, Ferguson RA. The influence of participant characteristics on the relationship between cuff pressure and level of blood flow restriction. *Eur. J. Appl. Physiol.* 2016;116:1421-1432.
- Jones DA, Bigland-Ritchie B, Edwards RHT. Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions. *Exp. Neurol.* 1979;64:414-427.
- Kacin A, Strazar K. Frequent low-load ischemic resistance exercise to failure enhances muscle oxygen delivery and endurance capacity. *Scand. J. Med. Sci. Sports.* 2011;21:e231-e241.
- Katayama K, Yoshitake Y, Watanabe K, Akima H, Ishida K. Muscle deoxygenation during sustained and intermittent isometric exercise in hypoxia. *Med. Sci. Sports. Exerc.* 2010;42:1269-1278.
- Kim S, Bosque J, Meehan JP, Jamali A, Marder R. Increase in outpatient knee arthroscopy in the United States: a comparison of National Surveys of Ambulatory surgery, 1996 and 2006. *J. Bone Joint Surg. Am.* 2011;93:994–1000.
- Kernell D, Monster AW. Motoneurone properties and motor fatigue. An intracellular study of gastrocnemius motoneurons of the cat. *Exp. Brain Res.* 1982;46:197–204.
- Laidlaw DH, Bilodeau M, Enoka RM. Steadiness is reduced and motor unit discharge is more variable in old adults. *Muscle Nerve* 2000;23(4):600-612.
- Laurentino GC, Loenneke JP, Teixeira EL, Nakajima E, Iared W, Tricoli V. The effect of cuff width on muscle adaptations after blood flow restriction training. *Med. Sci. Sports Exerc.* 2015;48(5):920-5.
- Lixandrão ME, Ugrinowitsch C, Berton R, Vechin FC, Conceição MS, Damas F, Libardi CA, Roschel H. Magnitude of Muscle Strength and Mass Adaptations Between High-Load Resistance Training Versus Low-Load Resistance Training Associated with Blood-Flow Restriction: A Systematic Review and Meta-Analysis. *Sports Med.* 2018;48(2):361-378.
- Ljubisavljevic M, Jovanovic K, Anastasijevic R. Changes in discharge rate of cat hamstring fusiform neurons during fatiguing contractions of triceps surae muscles. *Brain Res.* 1992;579:246-252.
- Loenneke JP, Pujol TJ. The use of occlusion training to produce muscle hypertrophy. *J Strength Cond. Res.* 2009;31:77–84.

- Loenneke JP, Thiebaud RS, Abe T. Does blood flow restriction result in skeletal muscle damage? A critical review of available evidence. *Scand. J. Med. Sci. Sports* 2014;24(6):415-422.
- Loenneke J P, Wilson JM, Wilson GJ, Pujol TJ, Bemben MG. Potential safety issues with blood flow restriction training. *Scand. J. Med. Sci. Sports* 2011;21(4):510-518.
- Millet GY, Aubert D, Favier FB, Busso T, Benoit H. Effect of acute hypoxia on central fatigue during repeated isometric leg extensions. *Scand. J. Med. Sci. Sports* 2009;19:695-702.
- Moritani T, Sherman WM, Shibata M, Matsumoto T, Shinohara M. Oxygen availability and motor unit activities in humans. *Eur. J. App. Physiol.* 1992;64:552-556.
- Nielsen JL, Aagaard P, Prokhorova TA, Nygaard T, Bech RD, Suetta C, Frandsen U. Blood flow restricted training leads to myocellular macrophage infiltration and upregulation of heat shock proteins, but no apparent muscle damage. *J. Physiol.* 2017;595(14):4857-4873.
- Pascoe MA, Holmes MR, Stuart DG, Enoka RM. Discharge characteristics of motor units during long-duration contractions. *Exp. Physiol.* 2014;99(10):1387-1398.
- Takarada Y, Takazawa H, Sato Y, Takebayashi S, Tanaka Y, Ishii N. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. *J. Appl. Physiol.* 2000;88(6):2097-2106.
- Taylor JL, Gandevia SC. A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *J. Appl. Physiol.* 2008;104:542-550.
- Taylor JL, Amann M, Duchateau J, Meeusen, Rice CL. Neural Contributions to Muscle Fatigue: From the Brain to the Muscle and Back Again.
- Tennant DJ, Hylden CM, Johnson AE, Burns TC, Wilken JM, Owens JG. Blood flow restriction training after knee arthroscopy: A randomized controlled pilot study. *Clin. J. Sport. Med.* 2017;27:245-252.
- Wernbom M, Augustsson J, Thomee R. Effects of vascular occlusion on muscular endurance in dynamic knee extension exercise at different submaximal loads. *J. Strength Cond. Res.* 2006;20:372-377.
- Westcott W. Strength training for frail older adults. *J. Active Aging* 2009;8:52-9.

Yasuda T, Brechue WF, Fujita T, Sato Y, and Abe T. Muscle activation during low-intensity muscle contraction with restricted blood flow. *J Sports Sci.* 2008;27(5):479-289.